Plasma progesterone response following ACTH administration during mid-gestation in the pregnant Brahman heifer

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Abstract

Previous reports of adrenal progesterone (P4) contributions during late gestation in cattle, and ACTH-induced P4 responses in the non-pregnant heifer, prompted a retrospective investigation to evaluate the plasma P4 response and the relative ratio of plasma cortisol (CT) to P4 following ACTH administration during mid-gestation in pregnant Brahman heifers. Twenty-three pregnant (139.0 ± 5.0 days of gestation) Brahman heifers received one of the following treatments: 0 (saline; n = 5), 0.125 (n = 4), 0.25 (n = 5), 0.5 (n = 4), or 1.0 (n = 5) IU of ACTH per kg BW. Blood samples were collected at −15 and −0.5 (time 0), 15, 30, 45, 60, 75, 105, 135, 165, 195, and 255-min post-ACTH challenge. Plasma P4 and CT were quantified by RIA. Pre-ACTH P4 did not differ (P > 0.10) among ACTH treatment groups (pooled, 12.1 ± 0.6 ng/mL). Among peak P4 values at 15-min post-ACTH infusion, control P4 (9.6 ± 1.2 ng/mL) tended to be lower (P < 0.07) than 0.5 IU ACTH-treated heifers (13.3 ± 1.1 ng/mL); and were lower (P < 0.02) than 0.25 and 1.0 IU ACTH-treated heifers (14.7 ± 1.1 and 22.2 ± 3.7 ng/mL, respectively). During the primary P4 response period (0 to 75-min post-ACTH), the area under the curve (AUC) was greater (P < 0.05) for 1.0 IU ACTH-treated heifers than all other groups. The CT:P4 ratios were lower (time × treatment, P < 0.01) for control heifers than all ACTH-treated heifers. Among ACTH-treated heifers, CT:P4 ratio response and CT:P4 ratio...
AUC were similar (P > 0.10) following ACTH challenge. In conclusion, acute increases in ACTH elevated plasma P₄, likely of adrenal origin, in mid-gestation pregnant heifers, while the CT:P₄ ratio (relative output) remained constant irrespective of ACTH dose (0.125–1.0 IU). Whether ACTH-induced increases in P₄ in pregnant animals are of physiological significance (e.g., an accessory role in the maintenance of pregnancy during periods of acute stress) remains to be determined.

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1. Introduction

The role of the adrenal gland and its potential contribution to reproductive function in mammals has been theorized for some time [1,2], with definitive roles of the fetal adrenal gland and cortisol (CT) linked to the initiation of parturition [3,4]. Further findings of the adrenal gland as a substantive source of progesterone (P₄) in rats [5,6], deer [7–9], ewes [10] and cows [11,12] have furthered speculation that the adrenal gland may serve as a supplemental source of P₄ in support of some aspects of reproduction. The actions of supplemental placental and/or fetal adrenal P₄ production have been shown to be supportive of pregnancy by suppressing the maternal immune system to prevent rejection of the developing fetus and placenta, as well as in ensuring myometrial quiescence for the maintenance of pregnancy (primate; [13]). In cattle, the administration of prostaglandin F₂α between 32 and 250 days of gestation reduce serum P₄, but abortion does not consistently occur beyond 150 days of gestation [14,15]. Moreover, bilateral ovariectomy before 200 days of gestation resulted in the termination of pregnancy [16,17], while pregnancy maintenance and successful parturition can occur if ovariectomy is performed after 200 days of gestation [17–19]. After 150 days of gestation, in conjunction with placental steroidogenesis, it has been suggested that small amounts of adrenal P₄ may contribute to maintenance of pregnancy in the absence of the ovaries [20,21].

In the rat, ACTH has been shown to stimulate P₄ production and possibly bind to a specific receptor in luteal tissue [22]. In contrast, in cattle ACTH has no direct effect on luteal P₄ synthesis as demonstrated by ovarian perfusion [23] and in vitro treatment of CL slices with ACTH [24]. Thus, increases in circulating P₄ concentrations following ACTH administration (or during times of acute stress) are most likely of adrenal origin. A direct effect of ACTH on adrenal P₄ production has been clearly demonstrated in the ovariecotized heifer [12]. As intact dairy heifers reached reproductive maturity [25], ACTH treatment significantly elevated serum P₄ concentrations. Of interest, however, is to what extent P₄ concentrations may increase (beyond that of luteal origin) in the pregnant animal following ACTH administration.

In the present study, mid-gestation pregnant Brahman heifers were treated with one of four doses of ACTH and serum P₄ concentrations were determined at various intervals post-challenge. Additionally, the relationship between circulating concentrations of CT relative to P₄ was also examined. This study was a retrospective study of samples collected from a previous investigation on ACTH dose–responses and the effects of transportation.
stress on pregnant Brahman cattle [26]. As the CT data have been reported previously, the current study focused solely on the $P_4$ response and its relationship to circulating CT concentrations in ACTH-treated and non-treated pregnant Brahman heifers.

2. Materials and methods

Twenty-three pregnant Brahman heifers (139.0 ± 5.0 days of gestation; 459.8 ± 9.2 kg) were randomly assigned to receive saline ($n = 5$) or one of four doses of ACTH: 0.125 ($n = 4$), 0.25 ($n = 5$), 0.5 ($n = 4$) or 1.0 ($n = 5$) IU of ACTH (Porcine ACTH #A6303, ACTH fragment 1–39; Sigma-Aldrich Chem. Co., St. Louis, MO) per kilogram BW. Jugular cannulas were established in each heifer 1-day before application of treatments to collect blood samples and administer ACTH. Approximately 15 cm of Teflon tubing (i.d. 1.1 mm, o.d. 1.7 mm; Teflon TFE, Cole-Parmer, Chicago, IL, USA) was inserted toward the heart into the jugular vein, with another 15-cm left outside the animal. The external end of the tubing was fitted with an 18-gauge stub adapter. A 2% Na–EDTA solution was introduced into the tubing, which was then sealed with a plastic cap that fit into the stub adapter. The external end of the tubing was taped to each cow’s neck until the next day, when 2.3-m of extension tubing (i.d. 1.65 mm, Tygon microbore, Cole-Parmer) was added before treatment.

On the day of treatment, heifers were placed in a chute system constructed of portable panels. Blood samples (10 mL) were drawn through the cannulas with a syringe containing 0.1 mL of a 10% Na-EDTA solution at 15 and 0.5 min (time 0) before, and at 15, 30, 45, 60, 75, 105, 135, 165, 195, and 255 min after ACTH treatment. Blood samples were placed immediately on ice and centrifuged (1800 × g) within 2-h of sampling. Plasma was harvested from samples following centrifugation and frozen at −20 °C until hormone analysis.

Plasma CT concentrations were determined in duplicate using commercially available RIA kits (Pantex, Santa Monica, CA, USA) validated for use in cattle [27,28]. The actual plasma CT response to the ACTH challenge described in this investigation has been published previously [26], with an intra-assay CV for CT of 12.4%. Plasma $P_4$ was determined by RIA using procedures validated previously in our laboratory [29], with an intra-assay CV of 8.6%. The plasma $P_4$ response and CT:$P_4$ ratio were normalized to pre-treatment (−15 and 0.5 min) values and data expressed as fold increases over baseline. Hormonal profiles were further analyzed using ANOVA for repeated measures (StatView; SAS Institute, Cary, NC, USA) as a split-plot with factors including sample interval and treatment × sample interval. Additionally, peak $P_4$ values at 15-min post-ACTH administration and the area under the primary response curve (Trapezoidal rule; 0 to 75 min and 75 to 255-min post-ACTH) were analyzed (one-way ANOVA) relative to treatment, and means (±S.E.) separated, using the Student’s $t$-test where appropriate. As indicated, for statistical analysis and descriptive purposes, a primary response period of 0–75 min was demarcated for primary analysis based on the return of concentrations of $P_4$ in all treatment groups to baseline values by 75-min post-ACTH (see Fig. 1 and inset), and the beginning of a segmentation of treatment effects in the dose-response of CT to ACTH treatment (i.e., in the duration of CT elevations post-ACTH; Fig. 2).
Fig. 1. Administration of ACTH to pregnant, mid-gestation Brahman heifers acutely elevates plasma concentrations of progesterone (P₄; mean ± S.E.). Data were further expressed as fold increases above respective pre-ACTH treatment values to normalize treatments (Fig. 1 inset). Among peak P₄ values at 15-min post-ACTH infusion, concentrations of P₄ for controls tended ($P < 0.07$) to be lower than 0.5 IU ACTH-treated heifers, and were lower ($P < 0.02$) than 0.25 and 1.0 IU ACTH-treated heifers.

Fig. 2. Administration of ACTH to pregnant heifers increases the plasma cortisol: progesterone (CT:P₄) ratio in all treatment groups, and decreases in a dose-dependent fashion. Data are expressed as fold increases above respective pre-ACTH treatment values to normalize treatments (animals). The CT:P₄ ratios were lower (time × treatment; $P < 0.01$) for control heifers than all ACTH-treated heifers. Among ACTH treated heifers, the CT:P₄ ratio primary response (0–75 min) was similar ($P > 0.10$) following ACTH challenge. After 75-min post-challenge, the area under the response curve increased with ACTH dose.
Table 1
Area under the primary response curve (AUC in relative units; 0–75-min post-challenge) for pregnant Brahman heifers treated with ACTH

<table>
<thead>
<tr>
<th>ACTH treatment (IU)</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Progesterone (P₄)</td>
</tr>
<tr>
<td>0</td>
<td>897.0 ± 75.1 a</td>
</tr>
<tr>
<td>0.125</td>
<td>945.0 ± 143.0 ab</td>
</tr>
<tr>
<td>0.25</td>
<td>1192.4 ± 76.8 b</td>
</tr>
<tr>
<td>0.50</td>
<td>1090.0 ± 88.3 ab</td>
</tr>
<tr>
<td>1.0</td>
<td>1558.6 ± 272.7 c</td>
</tr>
</tbody>
</table>

The different letters (a, b, c) that differ within column, \( P < 0.05 \).

3. Results

Administration of ACTH to pregnant Brahman heifers elevated plasma P₄ concentrations (Fig. 1). Data were further expressed for analysis as fold increases above respective pre-ACTH treatment values (pooled −15 and 0 min time-points) to normalize all animals to pre-ACTH baseline concentrations of P₄ (Fig. 1 inset). Among peak P₄ values, observed at 15-min post-ACTH infusion, plasma P₄ concentration for controls (no ACTH; 9.6 ± 1.2 ng/mL) tended to be lower \( (P < 0.07) \) than 0.5 IU ACTH-treated heifers (13.3 ± 1.1 ng/mL); and was lower \( (P < 0.02) \) than 0.25 and 1.0 IU ACTH-treated heifers (14.7 ± 1.1 and 22.2 ± 3.7 ng/mL, respectively). During the primary P₄ response period (0–75-min post-ACTH) depicted in Fig. 1, the area under the curve was greater \( (P < 0.05) \) for 1.0 IU ACTH-treated heifers than all other groups (Table 1). Area under the curve was also greater \( (P < 0.05) \) for the 0.25 IU ACTH group than the controls.

Administration of ACTH to pregnant heifers acutely increased the plasma CT:P₄ ratios in all treatment groups; they subsequently decreased in a dose-dependent fashion (Fig. 2). The CT:P₄ ratios were lower \( (time \times treatment; P < 0.01) \) for control heifers than for all ACTH-treated heifers (Fig. 2). Among ACTH-treated heifers, CT:P₄ ratio primary responses (0–75 min) were similar \( (P > 0.10) \) following ACTH administration. Similarly, the CT:P₄ ratio area under the primary response curve (AUC: 0–75 min) was greater \( (P < 0.05) \) for all ACTH-treated heifers compared to controls, and was similar \( (P > 0.10) \) among ACTH-treated heifers (Table 1). During the entire measurement period (0–255 min), a dose-dependent effect was evident \( (P < 0.05) \) in CT:P₄ ratios; as the dose of ACTH increased, so did the area under the response curve after 75-min post-ACTH challenge (Fig. 2).

4. Discussion

The overall function of the adrenal gland is to protect against stress, acute and chronic [30]. In some species, for example, cervids (white-tailed and fallow deer), it has been suggested that the secretion of adrenal P₄ during pregnancy may serve to support P₄-secreting luteal and/or placental tissues during times of acute stress, thus protecting the
fetus from abortion in the presence of high circulating concentrations of corticosteroids [7,31,32]. The output of P4 from the adrenal gland can be substantial, which in deer can be as much as two to five times more than maximum luteal output [7–9]. In the bovine, the responsiveness of the adrenal gland with respect to adrenal P4 production and its potential role in reproduction has not been elucidated completely. In the present investigation, treatment of mid-gestation pregnant heifers with ACTH acutely increased plasma concentrations of P4. As the bovine CL does not appear to be directly stimulated by ACTH [23,24], as it is in the rat [22], the source of the ACTH-induced elevations of P4 was most likely of adrenal origin, although the actual source (maternal adrenal, fetal adrenal or placental) cannot be determined. We suggest, as has been previously speculated by others [21], that this acute increase in P4 may act as a protective mechanism for pregnancy maintenance in the presence of any potentially deleterious effects of a stress-induced response. This might include maintaining integrity of the “progesterone block” on CT cascade-induced myometrial contractions to prevent pre-term labor, or for overcoming ACTH/CT inhibition (directly or indirectly) of gonadotropin release [33,34], which may affect luteal function.

The acute plasma P4 response following ACTH administration in this study peaked at 15-min post-challenge, which was similar to adrenal P4 responses observed in ACTH challenge studies of lactating dairy cows with follicular cysts; which peaked at 30-min post-challenge [35]. An increase in circulating concentrations of P4 following ACTH administration has also been observed in estrual heifers treated with ACTH in a gelatin vehicle, with peak P4 occurring around 2.6 h post-ACTH treatment [36]. These and many other studies have documented the potential of the adrenal gland to serve as a source of P4 in the bovine. Of note is the work of Wendorff et al. [21], in which studies of adrenalectomized-ovariectomized late gestation cows (>215 days of gestation) suggested a potential contribution of the maternal adrenal gland to the maintenance of pregnancy. The placenta has since been implicated as a major endocrine organ (albeit transient) responsible for maintenance of pregnancy in the late gestation cow; though supplemental contributions of the adrenal gland cannot be discounted or eliminated completely. Studies of pregnant and non-pregnant cows and heifers exhibiting elevated concentrations of P4 of apparent adrenal origin suggest that the adrenal gland may, in fact, play an important role in many facets of reproduction. However, as long-term ACTH exposure has produced inhibitory influences on reproductive processes in some species (cattle: [37]; rats: [38]), more studies aimed at delineating the role of the adrenal gland and the relevance of adrenal P4 contributions to reproductive function during acute and chronic exposure to stressors are needed [25].

Plasma CT relative to P4 increased similarly among ACTH-treated heifers, and decreased in a dose-dependent fashion. However, the amount of P4 produced following ACTH administration did not significantly alter the CT:P4 ratio during the primary response period in which P4 was acutely elevated in the 0.25, 0.5, and 1.0 IU ACTH groups (see Fig. 1 inset; note that while significant, only a 0.5-fold increase over baseline values was observed in the 1.0 IU ACTH treatment group). This would suggest that stimulation of steroidogenesis of the adrenal gland by ACTH produced concomitant increases in CT and P4, and not necessarily a shift from one steroid to the other in response to stimulation. In the biosynthesis of steroids in the adrenal gland, cholesterol
is converted to pregnenolone, which can then be directed toward synthesis of progesterone (via 3β-hydroxysteroid oxidoreductase and Δ5→4-3-oxosteroid isomerase) or 17-hydroxypregnenolone (via 17-hydroxylase). Either of these forms can be further converted to 17-hydroxyprogesterone, which can be directed toward the production of CT. The magnitude of the CT increase in this study was five- to six-fold greater [26] than the relative increase reported here for stimulated P4 production. Of note was the absence of a dose-response in peak plasma concentrations of CT [26], whereas peak plasma concentrations of P4 were clearly greatest in the 1.0 IU ACTH treatment group (15-min post-ACTH), with increases in the 0.25 and 0.5 IU ACTH groups over the control and 0.125 IU ACTH-treated heifers. One might speculate that as plasma concentrations of CT reached maximal stimulation with all doses of ACTH administered, that the acute increase in P4 following ACTH challenge might be due to a shuttling of steroidogenic substrates toward P4 production. Whether this is a consequence of limited enzymatic availability, an inherent stop-gap to prevent excessive CT production, or truly a mechanism for P4 supplementation in an acutely stressed animal (at least chemically in this model) remains to be seen. The prolonged CT response, which occurred in a dose-dependent fashion, following 75-min post-challenge (Fig. 2, [26]), is unclear given the relatively short half-life reported for ACTH (approximately 10 min; [26,39]). This could be due, in part, to a prolonged half-life of ACTH due to overwhelmed enzymatic and protease systems and/or prolonged binding of ACTH to cell-surface receptors [26]. Nevertheless, plasma P4 was essentially at pre-treatment baseline and control values by 75-min post-challenge and did not alter the dose-dependent nature of this lag effect when expressed as a CT:P4 ratio. Thus, the prolonged stimulatory response (from 75 to 255-min post-ACTH) appears more related to an issue of over-stimulation and/or clearance of CT from the system rather than any direct relationship to alterations or shifts toward adrenal P4 production.

Collectively, these data indicated that ACTH administration can acutely increase plasma P4 production above that of normal (basal) circulating concentrations of P4 in the mid-gestation Brahman heifer. These increases in plasma P4 were most likely of adrenal origin, although extra-adrenal sources cannot be ruled out completely. Whether the maternal adrenal gland in some species, including the bovine, plays an active role in the maintenance of pregnancy during periods of acute stress, or when luteal or placental P4 production becomes compromised, remains to be determined. Secondarily, as a diagnostic consideration, these data would further suggest that in acutely stressed animals, plasma concentrations of P4 could be higher than normal, thus confounding determinations made for concentrations of P4 during pregnancy in the bovine.

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